Acetic Acid, the Active Component of Vinegar, Is an Effective Tuberculocidal Disinfectant

Claudia Cortesia,[a] Catherine Vilchèze,[b] Audrey Bernut,[c] Whendy Contreras,[a] Keyla Gómez,[d] Jacobus de Waard,[d] William R. Jacobs, Jr.,[b] Laurent Kremer,[c,e] Howard Takiff[a]

Laboratorio de Genética Molecular, CMBC, IVIC, Caracas, Venezuela[a]; Howard Hughes Medical Institute, Albert Einstein College of Medicine, Bronx, New York, USA[b]; Laboratoire de Dynamique des Interactions Membranaires Normales et Pathologiques, CNRS UMR5235, Université de Montpellier 2, Montpellier, France[c]; Laboratorio de Tuberculosis, Instituto de Biomedicina, Universidad Central de Venezuela, Caracas, Venezuela[d]; INSERM, DIMNP, Montpellier, France[e]

ABSTRACT **Effective and economical mycobactericidal disinfectants are needed to kill both *Mycobacterium tuberculosis* and non-*M. tuberculosis* mycobacteria. We found that acetic acid (vinegar) efficiently kills *M. tuberculosis* after 30 min of exposure to a 6% acetic acid solution. The activity is not due to pH alone, and propionic acid also appears to be bactericidal. *M. bolletii* and *M. massiliense* nontuberculous mycobacteria were more resistant, although a 30-min exposure to 10% acetic acid resulted in at least a 6-$\log_{10}$ reduction of viable bacteria. Acetic acid (vinegar) is an effective mycobactericidal disinfectant that should also be active against most other bacteria. These findings are consistent with and extend the results of studies performed in the early and mid-20th century on the disinfectant capacity of organic acids.**

IMPORTANCE **Mycobacteria are best known for causing tuberculosis and leprosy, but infections with nontuberculous mycobacteria are an increasing problem after surgical or cosmetic procedures or in the lungs of cystic fibrosis and immunosuppressed patients. Killing mycobacteria is important because *Mycobacterium tuberculosis* strains can be multidrug resistant and therefore potentially fatal biohazards, and environmental mycobacteria must be thoroughly eliminated from surgical implements and respiratory equipment. Currently used mycobactericidal disinfectants can be toxic, unstable, and expensive. We fortuitously found that acetic acid kills mycobacteria and then showed that it is an effective mycobactericidal agent, even against the very resistant, clinically important *Mycobacterium abscessus* complex. Vinegar has been used for thousands of years as a common disinfectant, and if it can kill mycobacteria, the most disinfectant-resistant bacteria, it may prove to be a broadly effective, economical biocide with potential usefulness in health care settings and laboratories, especially in resource-poor countries.**

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Address correspondence to Howard Takiff, htakiff@gmail.com.

Mycobacteria are important agents of human infections. While the most well-known infections are tuberculosis and leprosy, infections with nontuberculous mycobacteria are an increasing problem in soft tissues after surgical or cosmetic procedures (1) or in the lungs of cystic fibrosis and immunosuppressed patients (2). Killing mycobacteria in health care settings is important because *Mycobacterium tuberculosis* strains in clinical specimens or cultures may contain drug-resistant organisms that are difficult or impossible to eradicate (3), and environmental mycobacteria must be thoroughly eliminated from surgical implements and respiratory equipment.

Disinfection of mycobacteria can be a problem, as mycobacteria are the most disinfectant-resistant bacteria (4). Quaternary ammonium compound (QAC) disinfectants are regarded as only mycobacteriostatic (4), and QAC tolerance is a problem with mycobacteria of the *Mycobacterium abscessus* complex (5). Some of the more effective disinfectants (6), such as chlorine bleach and peracetic acid, can be relatively toxic, unstable, or expensive (4), and the emergence of glutaraldehyde-resistant strains has caused extensive outbreaks of nontuberculosis infections (7).

While using acetic acid as a solvent, we fortuitously found that it had significant mycobactericidal activity. Vinegar has been used as a disinfectant for thousands of years (8, 9) and is today commonly used for, among other things, eliminating bacteria from fresh produce (10, 11) or curing acute otitis externa (12, 13). There is a description of its use in the Middle Ages for treating infected wounds and scrofula, which generally means tuberculosis of the lymph nodes in the neck (http://en.wikipedia.org/wiki/Medieval_medicine_of_Western_Europe). In this study, we quantitated the significant mycobactericidal activity of acetic acid and suggest that it may be a useful, low-cost, and effective disinfectant.

Acetic acid or vinegar was added to aliquots of bacterial cultures in proportions to create the concentrations shown in Table 1. Commercial white vinegar was used whenever possible. The cultures were then inverted several times and incubated at room temperature without agitation for the specified periods (20 to 30 min). They were then centrifuged, and the pellet was resuspended in Middlebrook 7H9 medium supplemented with oleic acid-albumin-dextrose-catalase (OADC). Aliquots of serial dilu-

TABLE 1 Bactericidal effect of increasing exposure times and acid concentrations

Acid and/or conditions	Solution characteristics[a]						Log$_{10}$ reduction of viable bacteria[b]											
	BSA and RBC concn (%)[c]	pH	pH with *M. abscessus*[d]	Molar concn	Exposure time (min)	Final concn (%)	*E. coli* DH5α	*E. coli* survivors	*M. smegmatis*	*M. smegmatis* survivors	*M. tuberculosis* mc^{2}7000	*M. tuberculosis* H37Rv	MDR *M. tuberculosis*	XDR *M. tuberculosis*	*M. abscessus*	*M. bolletii*	*M. massiliense* R[f]	*M. massiliense* S[f]
Acetic acid				**0.34**	**20**	**2.5**			3						2			
				0.40	**30**	**3**						4	4	3				
				0.54	**20**	**4**						3	4	3	3			
		2.45	2.62	**0.83**	**20**	**5**	**8.5**	**7**	**7**	**7**		3	4.5	3	4			
				0.83	**25**	**5**									5			
				0.83	**30**	**5**					**7**[e]				5			
		2.43	2.57	**1.00**	**25**	**6**			**9**[e]						5.5		4	2
				1.00	**30**	**6**			**8**[e]		**7**[e]	**8**[e]	**8**[e]	**8**[e]	**8**	5	3	3
		2.31	2.42	**1.33**	**30**	**8**						**8**[e]	**8**[e]	**8**[e]		**7**	4.5	5
		2.27	2.40	**1.67**	**30**	**10**			**8**[e]			**8**[e]	**8**[e]	**8**[e]		**6**	**6**	**6**
Acetic acid, dirty conditions	2				**20**	**6**			**6**									
	3				**25**	**6**			**7**									
	3.5				**25**	**7**			**9**[e]									
	2.5				**30**	**10**											**8**[e]	
Propionic acid				**0.9**	**20**	**6.7**	**8**[e]											
				1.08	**20**	**8.0**									**7**			
				1.08	**25**	**8.0**									**7**			
				1.15	**20**	**8.5**	**8**[e]											

[a] High-level disinfectant activity is indicated in bold.

[b] The numbers represent the reductions, expressed in log$_{10}$, in the number of colonies recovered after the acid exposures, compared to that of controls exposed to sterile water alone under the same conditions.

[c] BSA, bovine serum albumin; RBCs, human red blood cells.

[d] *M. abscessus* with optical density at 600 nm of 1 was diluted 1:10 in the acetic acid solutions.

[e] Limit of detection (no colonies recovered from exposed bacteria).

[f] R and S refer to the rough and smooth morphotypes of *M. massiliense.*

tions were plated on LB agar plates for *Escherichia coli* and Middlebrook 7H10 plates for mycobacteria, except for *Mycobacterium smegmatis*, which was plated on either medium with the same results. All experiments were performed at least twice, and the results were the same whether the bacterial pellets were resuspended in Middlebrook 7H9 medium-OADC or in a sodium hydroxide solution to neutralize the acetic acid. Negative controls with sterile water were included to determine the original number of viable bacteria.

Exposure to acetic acid at a concentration of 5% for 20 min reduced viable *Escherichia coli* DH5α and *M. smegmatis* mc^{2}155 bacteria by at least 7 log$_{10}$ (Table 1). In some experiments in which the initial culture had ~9 log$_{10}$ of bacteria/ml, there were rare surviving colonies of *E. coli* and *M. smegmatis*. When these colonies were grown in broth media and tested again, they showed no evidence of resistance or tolerance to acetic acid, and their exposure to 5% for 20 min again produced 7 log$_{10}$ of killing.

To test whether the low pH of acetic acid was responsible for the bactericidal effect, we exposed *M. smegmatis* and *E. coli* for 20 min to a dilute solution of HCl in water with pH 2.5, corresponding approximately to the pH of 5% acetic acid. No bactericidal effect was seen (data not shown). Other studies have looked at the effect of pH on mycobacterial growth by reducing the pH in the culture media to as low as pH 3 with HCl or pH 5 with citric acid and found that after a couple of days, the number of viable bacteria was reduced by only approximately 1 log$_{10}$ (14, 15).

Next, to determine whether the effect was specific for acetic acid, a two-carbon acid, we also tested solutions of the three-carbon propionic acid that had been diluted to have approximately the same molarity as 6% acetic acid. Propionic acid had bactericidal activity against both *E. coli* (Table 1) and *M. abscessus*. A 13.7% solution of sodium acetate, with about the same molarity as 10% acetic acid, produced less than a 1-log$_{(10)}$ killing of *M. smegmatis* (data not shown), indicating that the bactericidal effect of acetic acid was caused by its carboxylic acid function.

We then tested for activity against *M. tuberculosis*. While exposure to 5% acetic acid for 20 min obtained only a 3- to 4-log$_{10}$ reduction of viable bacteria, exposure to a 6% solution for 30 min resulted in at least an 8-log$_{10}$ reduction. The same levels of mycobactericidal activity were seen with virulent (H37Rv) and avirulent auxotrophic (mc^{2}7000 [H37Rv Δ*RD1* Δ*panCD*]) (16) *M. tuberculosis* laboratory strains, as well as with multidrug-resistant (MDR) and extensively drug-resistant (XDR) *M. tuberculosis* clinical isolates.

We also tested acetic acid on the notoriously resistant *Mycobacterium abscessus* complex bacteria (17). Although *M. abscessus sensu stricto* was efficiently killed by exposure to 6% acetic acid for 30 min, *Mycobacterium bolletii* and *Mycobacterium massiliense* were more resistant. The levels of reduction in viable bacteria differed somewhat in different experiments, but exposure to 10% acetic acid for 30 min achieved a minimum of a 6-log$_{10}$ reduction in the colony counts of these two species, with *M. massiliense* appearing to be slightly more resistant than *M. bolletii.*

Finally, we demonstrated that the mycobactericidal activity remained even under "dirty" conditions that were meant to simulate contamination with organic material in clinical samples, with the acetic acid solution containing 2 to 3.5% bovine serum albumin and 2 to 3.5% red blood cells (Table 1).

The results described above suggest that acetic acid can be an effective, economical bactericidal agent for *M. tuberculosis* and

nontuberculous mycobacteria, although the 20- to 30-min exposure time required to obtain optimal killing is longer than the 5 min recommended for some commercial bactericides. Exposure to 6% acetic acid for 30 min resulted in an 8-$\log_{10}$ reduction of viable *M. tuberculosis* bacteria, including XDR and MDR strains. In tests with the *M. abscessus* complex, which are emerging as the most pathogenic of the nontuberculous mycobacteria (17), the same conditions produced 8 $\log_{10}$ of killing only with *M. abscessus sensu stricto*, while *M. massiliense* and *M. bolletii* were more resistant in some assays. However, the generally accepted definition of an effective mycobactericide is one that has the ability to reduce viable bacteria by 4 to 5 $\log_{10}$ (18), while the 6-$\log_{10}$ reduction of both these bacteria achieved with 10% acetic acid for 30 min would be classified as high-level mycobactericidal activity (19). In some assays in which 10^9 *M. smegmatis* bacteria were exposed to 6% acetic acid for 20 min and 3×10^9 *M. massiliense* bacteria were exposed to 10% acetic acid for 30 min, there were no survivors. Although we did not attempt carrier tests, the effective mycobactericidal activity was maintained even under "dirty" conditions that simulate contamination with organic material. While this level of killing may not be adequate for all critical uses, such as sterilizing surgical instruments, it is likely that higher levels of killing of these highly resistant strains could be achieved with higher concentrations of acetic acid and/or longer exposure times than the ones tested here. Preliminary studies, however, suggest that 10% acetic acid for 30 min does not kill *Bacillus subtilis* spores (data not shown), so it cannot be classified as a high-level general disinfectant (20). We have not assessed the activity of acetic acid against viruses and fungi.

Although the disinfectant properties of organic acids such as acetic acid, propionic acid, and butyric acid—a component of sweat (21)—are well known, they are not usually included in reviews of bactericides (4). However, in the early part of the 20th century, they were fairly extensively studied for disinfectant properties, as reviewed by Reid in 1932 (22), and their tuberculocidal activity was evaluated by Barker in 1964 (23). It was found that they had broad bactericidal activity that increased with increasing carbon chain length through caprylic acid ($C = 8$), although the longer-chain acids were less soluble. Bactericidal activity was also found to increase with decreasing surface tension of the organic acid solutions and appeared to be due to the undissociated acid rather than the hydrogen ion concentration. It was therefore suggested that the bactericidal effect might be related to the ability of the acids to pass through the bacterial membrane (23).

Acetic acid is not very toxic, although prolonged exposure will produce corrosive effects, both on skin and metals. In reports from nearly 100 years ago, it was found that the topical application of a 1% acetic acid solution in saline cured *Pseudomonas aeruginosa* (*Bacillus pyocyaneus*) wound infections (24, 25). It might be worthwhile testing its effectiveness as a topical agent on mycobacterial ulcers (26, 27).

Acetic acid is relatively inexpensive—2.5 liters of 99% acetic acid costs less than US$100 and could effectively disinfect up to 20 liters of *M. tuberculosis* cultures or sputa. Commercial vinegar bought in supermarkets was used wherever possible in the experiments described here, but the concentrations vary from country to country. Commercial vinegar could be used at effective concentrations for *M. smegmatis* or *M. tuberculosis* in France, where it is sold as 8% acetic acid, but not in the United States or Venezuela, where vinegar is sold as 5% acetic acid. While longer-chain organic acids may have better bactericidal activity, acetic acid (vinegar) is relatively nontoxic, inexpensive, and available, which could make it an effective, economical biocide for disinfecting *M. tuberculosis* from clinical specimens, cultures, and laboratory surfaces, and it would be particularly useful in low-income countries. The high-level capacity of acetic acid in killing mycobacteria, regarded as the most disinfectant-resistant bacteria due to the structure of their lipid-rich cell walls (4), suggests that perhaps it should be revived as a broadly effective bactericide that can be used as a general sanitizer.

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